# Molecular Pathophysiology of Acid-Base Disorders

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Abstract: Acid-base balance is critical for normal life. Acute and chronic disturbances impact cellular energy metabolism, endocrine signaling, ion channel activity, neuronal activity, and cardiovascular functions such as cardiac contractility and vascular blood flow. Maintenance and adaptation of acid-base homeostasis are mostly controlled by respiration and kidney. The kidney contributes to acid-base balance by reabsorbing filtered bicarbonate, regenerating bicarbonate through ammoniagenesis and generation of protons, and by excreting acid. This review focuses on acid-base disorders caused by renal processes, both inherited and acquired. Distinct rare inherited monogenic diseases affecting acid-base handling in the proximal tubule and collecting duct have been identified. In the proximal tubule, mutations of solute carrier 4A4 (SLC4A4) (electrogenic Na/HCO-cotransporter Na/bicarbonate cotransporter e1 [NBCe1]) and other genes such as CLCN5 (Cl/H-antiporter), SLC2A2 (GLUT2 glucose transporter), or EHHADH (enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase) causing more generalized proximal tubule dysfunction can cause proximal renal tubular acidosis resulting from bicarbonate wasting and reduced ammoniagenesis. Mutations in adenosine triphosphate ATP6V1 (B1 H-ATPase subunit), ATPV0A4 (a4 H-ATPase subunit), SLC4A1 (anion exchanger 1), and FOXI1 (forkhead transcription factor) cause distal renal tubular acidosis type I. Carbonic anhydrase II mutations affect several nephron segments and give rise to a mixed proximal and distal phenotype. Finally, mutations in genes affecting aldosterone synthesis, signaling, or downstream targets can lead to hyperkalemic variants of renal tubular acidosis (type IV). More common forms of renal acidosis are found in patients with advanced stages of chronic kidney disease and are owing, at least in part, to a reduced capacity for ammoniagenesis.

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# Molecular pathophysiology of acid-base disorders

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**Conflict of Interest**

The authors report no conflict of interest.

**ABSTRACT**

Acid-base balance is critical for normal life. Acute and chronic disturbances impact on cellular energy metabolism, endocrine signaling, ion channel activity, neuronal activity, and cardiovascular functions such as cardiac contractility and vascular blood flow. Maintenance and adaptation of acid-base homeostasis are mostly controlled by respiration and kidney. The kidney contributes to acid-base balance by reabsorbing filtered bicarbonate, regenerating bicarbonate through ammoniagenesis and generation of protons, and by excreting acid. This review focuses on acid-base disorders caused by renal processes, both inherited and acquired. Distinct rare inherited monogenic diseases affecting acid-base handling in the proximal tubule and collecting duct have been identified. In the proximal tubule, mutations of SLC4A4 (electrogenic $Na^+/HCO_3^-$-cotransporter NBCe1) and other genes like CLCN5 ($Cl^-/H^+$-antiporter), SLC2A2 (GLUT2 glucose transporter) or EHHADH (enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase) causing more generalized proximal tubule dysfunction can cause proximal renal tubular acidosis due to bicarbonate wasting and reduced ammoniagenesis. Mutations in ATP6V1 (B1 $H^+$-ATPase subunit), ATPV0A4 (a4 $H^+$-ATPase subunit), SLC4A1 (anion exchanger 1) and FOXI1 (forkhead transcription factor) cause distal renal tubular acidosis (dRTA type I). Carbonic anhydrase II (CAII) mutations affect several nephron segments and give rise to a mixed proximal and distal phenotype. Last, mutations in genes affecting aldosterone synthesis, signaling or downstream targets can lead to hyperkalemic variants of renal tubular acidosis (type IV). More common forms of renal acidosis are found in patients with advanced stages of chronic kidney disease and are, at least in part, due to a reduced capacity for ammoniagenesis.

## Importance to maintain acid-base homeostasis

Acid-base homeostasis is critical for normal life. Both acute or chronic disturbances can have major impact on normal cellular and organ function and severe derangements can eventually lead to death. Acidosis alters ion channel function, neuronal excitability with reduced activity, cardiac arrhythmias, decreased cardiac contractility which together with vascular relaxation can cause hypotension. Acidosis impacts on metabolism by inducing insulin resistance, it inhibits glycolysis and induces resistance to catecholamines [1,2]. Chronic acidosis causes muscle wasting, contributes to development or exacerbation of bone disease, increases inflammation, and plays a role in the progression of CKD [3,4]. Alkalosis induces vasoconstriction with cardiac arrhythmias, confusion, and seizures from arteriolar vasoconstriction with decreased cerebral or myocardial perfusion and an increased affinity for $O_2$ to hemoglobin/reduced release of $O_2$ from hemoglobin. High pH also increases binding of ionized calcium to albumin [5].

## Coordinated regulation of acid-base homeostasis

Due to its vital importance, several mechanisms exist and cooperate to maintain and regulate systemic acid-base balance. Respiration contributes to acute and chronic acid-base balance by responding to changes in pH and/or $pCO_2$. Respiration adapts within minutes when pH or $pCO_2$ change [6-8]. Kidneys respond with a lag time of 2-4 hours with increased urinary acidification and acid excretion when facing an acid load [6]. Increased renal ammoniagenesis depends on sufficient supply of glutamine that in part is shuttled from liver and skeletal muscle contributing to muscle wasting in chronic acidosis [3,4]. Chronic acidosis may also impact on bone stability as bone is a reservoir for bicarbonate and phosphate and low pH can stimulate osteoclast activity [9].

## Renal processes required for normal acid-base balance

The kidneys play a central role in the long-term maintenance of acid-base homeostasis. The excretion of acid and the regeneration of approximately 1 mmol bicarbonate per kg bodyweight per day (e.g. 70 mmoles in an average person of 70 kg body weight per day) are critical tasks.

Maintenance and control of systemic acid-base balance by the kidney is based on three major processes: 1) the reclamation of filtered bicarbonate, 2) the excretion of acid mostly as ammonium and titratable acidity, and 3) the *de novo* synthesis of bicarbonate replacing bicarbonate used by metabolism [10].

The kidneys filter about 180 litres/day of primary urine containing an approximately 4500 mEq bicarbonate which in a healthy person in steady-state and with an average mixed diet is completely reabsorbed along the nephron. About 80% of the filtered bicarbonate is reabsorbed in the proximal tubule. In the first step protons are secreted by NHE sodium-proton exchangers (mostly the NHE3/*SLC9A3* isoform) and proton pumps ($H^+$-ATPases). The luminal activity of carbonic anhydrases (Carbonic anhydrase type IV (CAIV)) facilitates the formation of $CO_2$ and $H_2O$ from filtered $HCO_3^-$ and secreted $H^+$. Then, $CO_2$ and $H_2O$ diffuse into proximal tubule cells where this process is reversed by type II cytosolic carbonic anhydrase (CAII). The produced $HCO_3^-$ is released into blood via the basolaterally located sodium-bicarbonate cotransporter (NBCe1/*SLC4A4*) whereas protons can be recycled into urine across the luminal membrane (**Figure 1**). Recently an additional mechanism of proximal tubule bicarbonate reabsorption was suggested: reabsorption via an apically located sodium-dependent bicarbonate cotransporter (NBCn2) [11]. The significance of this transporter remains to be established. Moreover, a fraction of bicarbonate is

reabsorbed through the paracellular pathway in the proximal tubule driven by the luminal accumulation of chloride and the resulting lumen-negative potential.

The remaining bicarbonate (approx. 20 % of the filtered load) is subsequently reabsorbed along the thick ascending limb of the loop of Henle by transcellular mechanisms similar to the proximal tubule involving NHE exchangers and to a lesser extent $H^+$-ATPases on the luminal side and a $Cl^-/HCO_3^-$-exchanger at the basolateral side [12-14].

Metabolic processes consume bicarbonate (i.e. in the urea cycle) and produce acids (i.e. in amino acid metabolism) that must be buffered by bicarbonate. The kidney replenishes bicarbonate by *de novo* generation of bicarbonate during proximal tubular ammoniagenesis and by hydration of $CO_2$ in acid-secretory type A intercalated cells. Ammoniagenesis occurs in the proximal tubule through a series of enzymatic reactions located partially in the mitochondria and partially in the cytosol (**Figure 2, upper panel**). Glutamine is the primary fuel for ammoniagenesis and is mostly extracted from peritubular blood vessels. Glutamine is also absorbed from primary urine but since glutamine reabsorption is nearly 100 % of the filtered load, increased demand for glutamine during acidosis cannot be met and basolateral glutamine uptake is upregulated during acidosis. The transporter(s) mediating basolateral glutamine uptake have not been fully identified. The SNAT3 (SLC38A3) glutamine transporter is located basolaterally in the proximal tubule and its expression is enhanced during metabolic acidosis or hypokalemia, conditions stimulating ammoniagenesis and glutamine extraction [15-18]. Moreover, a mouse model with complete lack of Snat3 expression dies early but shows signs of reduced ammoniagenesis [19]. In contrast, a more recent study in mice deficient for the transcription factor Nrf2 showed that expression and regulation of Snat3 in kidney is greatly reduced in the absence of Nrf2 but that Nrf2 KO mice retain the full capacity to stimulate ammoniagenesis and urinary

ammonium excretion [20]. Thus, SNAT3 is likely not the only glutamine importing transporter. The amino acid exchanger LAT2 may also contribute.

Urinary ammonium excretion is a process involving several steps. In the proximal tubule, a fraction of ammonium is transferred into urine (another fraction is also released back into circulation). Then ammonium is mostly reabsorbed by the Na/K/2Cl-cotransporter NKCC2 in the thick ascending limb of the loop of Henle, accumulates in the (medullary) interstitium and is finally actively secreted by the cells lining the collecting duct system into urine in the form of ammonia (see below) (**Figure 2, lower panel**). Accumulation in the medullary interstitium requires also the NHE4 $Na^+/H^+$-exchanger (SLC9A4), the NBCn1 electroneutral $Na^+/HCO_3^-$-cotransporter (SLC4A7) and sulfatides [21-23].

Final urinary acidification and fine-tuning of renal acid-excretion is mediated by the collecting system consisting of the connecting tubule, the cortical and medullary parts of the collecting duct [24]. However, the first intercalated cells appear already in the late distal convoluted tubule [25]. At least two distinct subtypes of intercalated exist: type A intercalated cells and non-type A intercalated cells. The latter may exist in two different forms, as type B intercalated cells and as non-A/non-B intercalated cells. Whether these forms are only distinct states of the same cell type or represent two truly different cells has not been clarified up to date.

Acid-secretory type A intercalated cells mediate ammonia excretion into urine as well as urinary acidification coupled to *de novo* synthesis of bicarbonate (**Figure 3**). $CO_2$ hydration is facilitated by cytosolic carbonic anhydrase II (CAII) forming protons and bicarbonate [10]. Bicarbonate is released into blood via the basolaterally located chloride-bicarbonate exchanger AE1 (Anion exchanger 1, SLC4A1) whereas protons are secreted into urine by vacuolar-type $H^+$-ATPase pumps at the luminal membrane [24,26]. As discussed below, genetic mutations in SLC4A1 or two different subunits of the

multimeric $H^+$-ATPase (consisting of more than 14 subunits with multiple isoforms) cause inherited forms of distal renal tubular acidosis (dRTA) [27-29]; The secretion of protons into urine acidifies urine to a maximal pH of approximately 4.5 - 4.0. Stronger acidification of urine is impossible as $H^+$-ATPases must pump against a steep proton gradient (intracellular pH 7.2, luminal pH 4.5). Eventually, one liter of unbuffered urine of pH 4.5 contains about 30 μmoles protons, a negligible amount compared to the requirement to excrete 70 mmoles of acid. Several urinary buffers, so-called titratable acidity (the term refers to the method to measure titratable acidity by back-titrating acidified urine), buffer protons and thereby enhance the amount of excretable acid. The main “titratable acid» is phosphate, but creatinine and urate also contribute to variable extents. The sum of urinary ammonium plus titratable acidity minus bicarbonate is called net acid secretion. For the sake of simplicity, urinary phosphate can be taken as approximation for titratable acidity and urinary bicarbonate can be neglected when urine pH is below pH 6.5 [8].

Proton and ammonia secretion is coupled where luminal ammonia ($NH_3$) captures free protons and is trapped in urine in the form of ammonium ($NH_4^+$). Ammonia secretion by intercalated cells (and also by neighboring principal cells) is mediated by two related gas channels belonging to the family of the rhesus blood group proteins, namely RhBG and RhCG (**Figure 3**) [30-32]. RhBG is exclusively located at the basolateral membrane whereas RhCG is the only known apical transport pathway for ammonia [31,33]. RhCG is also found at the basolateral membrane where it also contributes to ammonia uptake from interstitium [31]. Rhcg and Rhbg are upregulated during acidosis in mice [34]. On the contrary, lack of Rhcg causes a form of dRTA in mice characterized by the inability to excrete ammonium [30,31,35,36]. The role of RhBG is less clear as a mild defect has been reported in one knockout mouse line but not in another [37-39]. Absence of Rhcg in mice is also associated with a mild defect in the capacity to

maximally acidify urine which may be caused by the stimulatory interaction of Rhcg with $H^+$-ATPases [40]. Intronic single nucleotide polymorphisms in RhCG have been associated with 24-hrs urinary pH but no mutations have been linked to human dRTA up to date [41]. The activity of type A intercalated cells and net acid secretion is stimulated during acidosis and reduced during alkalosis [10].

Type B intercalated cells are located in the distal convoluted tubule, connecting tubule and cortical collecting duct [25]. Only few type B intercalated cells are found in the initial part of the outer medullary collecting duct but are absent from the inner part of the outer medullary collecting duct and from the inner medullary collecting duct [25]. These cells express the chloride/bicarbonate exchanger pendrin (SLC26A4) on their luminal membrane, cytosolic carbonic anhydrase II, and $H^+$-ATPases on their basolateral membrane. Their main function is the secretion of bicarbonate to compensate for alkalemic states [42,43]. Non-type A/non-type B intercalated cells are characterized by the luminal appearance of pendrin and the colocalization of $H^+$-ATPases at the same membrane (and sometimes also additional $H^+$-ATPases at the basolateral membrane). Thus, in this cell type bicarbonate secretion by pendrin is paralleled by $H^+$-ATPase mediated proton secretion resulting in net chloride absorption [42,44]. Moreover, type B intercalated cells may play an important role in NaCl homeostasis by contributing to chloride absorption involving pendrin and the $Na^+$-dependent $Cl^-/HCO_3^-$-exchanger [45-51].

**Inherited disorders of renal acid-base handling**

Inherited disorders of renal acid-base handling are considered to be rare. Whether common polymorphisms or milder mutations may contribute to forms of incomplete dRTA and may predispose to nephrolithiasis or –calcinosis is not well

established. However, variants in some genes discussed below have been found in families with stone disease [52,53]. Clearly, larger and more detailed studies are warranted to elucidate the relevance of common variants.

### *Gene defects underlying proximal renal tubular acidosis (pRTA, type II RTA)*

#### *kNBCe1 (SLC4A4)*

Pathogenic mutations in the SLC4A4 gene encoding for the basolateral electrogenic sodium-bicarbonate cotransporter NBCe1 cause proximal renal tubular acidosis with ocular abnormalities and mental retardation (OMIM #604278, table 1) [54,55]. Patients often present with growth retardation and short stature, metabolic acidosis with low bicarbonate, mental retardation, teeth enamel defects, and bilateral glaucoma, cataracts, and band keratopathy [56]. Since proximal tubular bicarbonate transport is impaired the threshold for bicarbonate reabsorption is reduced and can be tested using an alkali-loading test restoring plasma bicarbonate levels to normal. Urinary acidification and ammoniagenesis are intact and due to metabolic acidosis will be maximized [57]. Other functions of the proximal tubule such as glucose, phosphate, or amino acid reabsorption are not primarily affected. Mutations identified are mostly missense mutations that affect trafficking of mutant transporters to the membrane and/or alter function of the transporter, e.g. reduced activity or inducing anion leaks [55,58,59].

Activity of NBCe1 is thought to be closely coupled to apical $Na^+/H^+$-exchanger activity, mostly mediated by the NHE3 isoform (SLC9A3 gene). Recently, several families with SLC9A3 mutations were identified for suffering from chloride diarrhea but

no renal or acid-base related phenotype was reported [60]. Mice with deletion of Slc9a3 are acidotic and show greatly reduced proximal tubule bicarbonate reabsorption [61].

***Fanconi-syndrome***

pRTA can be part of a generalized dysfunction of the proximal tubule known as De-Toni-Debré-Fanconi syndrome. Patients suffer from urinary loss of various substrates typically reabsorbed by the proximal tubule including bicarbonate, amino acids, glucose, low molecular weight protein, and phosphate. This syndrome can be acquired, e.g. due to drug toxicity or light chain disease [62,63], or inherited due to mutations in genes affecting overall proximal tubule function [64]. Various monogenic causes have been identified to underlie forms of the De-Toni-Debré-Fanconi syndrome including CLCN5 and ORCL (Dent's disease I and II), cystosin (CTNS) in cystonosis, ATP7B in Wilson disease, Fumarylacetoacetate hydrolase (FAH), Tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPD) in tyrosinemia type I-III, and Galactose-1-phosphate uridylyl-transferase (GALT) in galactosemia (table 1).

***Gene defects underlying distal renal tubular acidosis (dRTA, type I RTA)***

Typical dRTA is characterized by the inability to acidify urine below pH 5.3 in the presence of metabolic acidosis. Urinary excretion of ammonium and titratable acids is also decreased causing an overall reduction in urinary acid excretion [10]. Patients present with hyperchloremic metabolic acidosis with normal anion gap often associated with hypokalemia. Failure to thrive, growth retardation, rickets, and nephrolithiasis or nephrocalcinosis may occur during childhood and adolescence and often lead to the initial diagnosis. Some patients also develop polyuria which may be

triggered by the reduced capacity to concentrate urine because of hypercalciuria, hypokalemia or nephrocalcinosis [49,65,66]. Mutations in at least 4 different genes can cause distal renal tubular acidosis (dRTA) in humans: SLC4A1, ATP6V0A4, ATP6V1B1, FOXI1 (table 1). Likely mutations in additional genes contribute to the pathogenesis of dRTA as about 20-30 % of patients with inherited forms of dRTA have no mutations in the coding regions of these 4 genes. Additionally, mutations in non-coding regions of these known genes such as in the promoter region may also cause dRTA but this has not been systematically addressed to date.

***SLC4A1 (AE1)***

SLC4A1 encodes for the chloride-bicarbonate exchanger Anion Exchanger 1 (AE1), in kidney specifically expressed in type A intercalated cells. Two transcripts exist with a longer transcript encoding for the erythroid AE1 variant whereas a shorter transcript encodes for the renal variant that lacks the first 65 amino acids. Mutations in SLC4A1 are repsonsible either dRTA or red blood cell abnormalities including spherocytosis or South-East Asian ovalocytosis (SAO). Most mutations cause either dRTA or hematological abnormalities but only few mutations are associated with both diseases. Inheritance is usually autosomal dominant but few autosomal recessive mutations have been described. The most frequent recessive mutation, G701D, causes dRTA that can be associated with hemolytic anemia. AE1 interacts with the chaperone glycophorin which is only expressed in red blood cells and is able to rescue "renal" mutations bringing them to the red blood cell membrane [67]. A series of additional mutations has been identified that are more common in South-East Asia and are frequently associated with a red blood cell phenotype. It has been hypothesized that some of these mutations may confer resistance to malaria [68]. In contrast to the

recessive mutations, patients with a Caucasian background carry more frequently dominant mutations, the R589H being the most common one, that rather causes dRTA [69,70]. Several classes of AE1 mutations have been identified that may cause either intracellular retention of mutant AE1 or even mistargeting to the luminal membrane of intercalated cell models [71-73]. In mice, the complete absence of AE1 causes severe metabolic acidosis and reduced renal acid excretion [74]. Introduction of the R607H mutation in mice (corresponds to human R589H) impairs intercalated cell function and reduces expression of proton pumps [69].

### *ATP6V1B1 (B1 $H^+$-ATPase) and ATP6V0A4 (a4 $H^+$-ATPase)*

Mutations in the genes encoding for the B1 (ATP6V1B1) and a4 (ATP6V0A4) $H^+$-ATPase subunits cause dRTA [28,75]. The $H^+$-ATPase proton pump is a multimeric protein complex with two major domains, the cytosolic catalytic $V_1$-domain hydrolyzing ATP (with 8 subunits A-H) and the membrane-bound $V_0$-domain mediating proton transfer with the a, c, c", d, and e subunits [76]. The B1 subunit is part of the $V_1$-domain whereas the a4 subunit belongs to the $V_0$-domain (**Figure 4**). The B1 subunit is selectively expressed in kidney, inner ear, epididymis and lung. In kidney, the B1 subunit is highly enriched in all subtypes of intercalated cells but is also found at lower levels in the thick ascending limb of Henle. Similarly, the a4 subunit is also enriched in all types of intercalated cells but is also highly abundant in the proximal tubule and in the thick ascending limb of the loop of Henle [77]. The subunit is also expressed in epididymis and the stria vascularis of the inner ear [75,78]. The expression of both subunit isoforms, B1 and a4, in the inner ear explains the occurrence of sensorineural deafness in patients with mutations in these subunits. However, the progression of sensorineural deafness is variable in patients and is not responsive to alkali therapy [79,80]. Some

patients also develop dizziness likely secondary to an enlarged vestibular aqueduct (EVA) [80]. Whether male fertility is altered due to alterations in proton pump function in the epididymis has not been examined.

Experiments in yeast and cell culture models demonstrated that most of the B1 subunit mutations cause either dysfunction or impaired assembly of the protein complex [81,82]. Mice deficient for the B1 subunit have a reduced capacity to acidify urine and develop more severe metabolic acidosis when acid-challenged. When bred with hypercalciuric mice, B1 knockout mice develop severe nephrocalcinosis with hydronephrosis [65,83,84]. Also, proton pumps lacking the B1 subunit do not respond to stimulation by angiotensin II [84].

Deletion of the a4 subunit in mice leads to severe dRTA with hypokalemia, nephrocalcinosis, and lower bone mineral density [85,86]. a4 knockout mice develop also massive hearing loss and a reduced sense of smell. The a4 subunit is also expressed in the proximal tubule and its absence is associated with low molecular weight proteinuria demonstrating an important role of this subunit in receptor-mediated endocytosis [86]. In one series of patients with mutations in either *ATP6V1B1* or *ATP6V0A4*, mutations in the latter were associated with a more severe clinical presentation and reduced kidney function [86]. However, another series did not find a clear association between mutations in ATP6V1B1, ATP6V0A4, or SLC4A1 and progression to CKD [70]. Of note, many patients had signs of a Fanconi-syndrome at initial presentation which ceased upon alkali therapy [70].

### *FOXI1*

More recently, missense mutations in the forkhead transcription factor FOXI1 were identified in two families with hyperchloremic non-anion gap dRTA and sensorineural

deafness [87]. Mutations identified are missense mutations that impair the ability of FOXI1 to activate its target genes whereas translocation into the nucleus appeared to be intact. In kidney, transcriptional targets of FOXI1 are the AE1 and AE4 anion exchangers, and the A, B1, E2 and a4 subunits of the $H^+$-ATPase [88-90]. Deletion of Foxi1 in mice causes dRTA with deafness and infertility in male mice. FOXI1 is expressed in mouse kidney, in the epithelium of the endolymphatic duct and sac epithelium of the inner ear [88-90]. In mouse kidney, absence of Foxi1 causes loss of differentiation of the different cell types in the collecting duct giving rise to undifferentiated cell type coexpressing markers of principal and intercalated cells [89]. Likewise, in Xenopus frog skin, the formation of mitochondria-rich proton-secreting cells (resembling collecting duct intercalated cells) depends on FOXI1 activity [91].

***WDR72***

Mutations in WDR72 have been identified in a few fami-lies with amelogenesis imperfecta, a disease reducing min-eralization of teeth. Some of the affected individuals were also tested for renal function and had metabolic acidosis and impaired urinary acidification suggesting the presence of dRTA. Mutations in WDR72 appear to be inherited in an autosomal recessive manner. The hearing status of affected patients is unknown. Single cell transcriptome data from mouse kidney suggest a high enrichment of Wdr72 in the collecting duct but the exact function of the protein is currently unknown. Of note, GWAS linked WDR72 to urinary pH and risk for lower eGFR [92-95].

### *Further genes*

Not all cases of inherited dRTA can be explained by mutations in the coding region of the genes discussed above suggesting that additional mutations may lie in the non-coding region, e.g. promoter region, or that mutations in additional genes may contribute to inherited dRTA. Candidate genes include the $K^+/Cl^-$-cotransporter KCC4 (SLC12A7) [96], the $Cl^-/HCO_3^-$ -exchanger SLC26A7 [97], the ammonia channels RhBG and RhCG (SLC42A2, SLC42A3) [30], the hensin (DMBT1)-CXCL12 signal complex [98,99], or other $H^+$-ATPase subunits [100].

## *Gene defects with mixed proximal and distal renal tubular acidosis*

Mixed RTA with features of proximal and distal defects in bicarbonate reabsorption and urinary acid excretion is seen in patients with mutations in carbonic anhydrase II [101] (table 1). In addition, patients suffer from osteopetrosis and mental retardation due to functions of CAII in bone and brain. The combined renal defect is explained by the central role of CAII in proximal bicarbonate transport and distal generation of $H^+$ and $HCO_3^-$ (see figures 1 and 3).

## *Gene defects causing hyperkalemic dRTA (type IV dRTA)*

Hyperkalemic dRTA is caused by gene defects in the pathways underlying aldosterone synthesis, signaling or its targets (table 1). Aldosterone is involved in regulating collecting duct acid-base transport [10,102] and is critical to maintain normokalemia [103,104]. Hyperkalemia as a consequence of aldosterone deficiency by itself may suppress ammoniagenesis and urinary acidification [102,105].

Inactivating mutations of 11-beta-hydroxylase (aldosterone synthase) reduce aldosterone levels whereas loss-of-function mutations in the mineralocorticoid receptor (NR3C2) impair aldosterone signaling [106,107]. In contrast, mutations in the three subunits of the epithelial $Na^+$-channel (ENaC) can underlie forms of pseudohypoaldosteronism with renal salt loss, hyperkalemia, and acidosis (table 1). Also mutations in WNK1 and WNK4 as well as in Kelch-like3 (KLHL3) and Cullin (CUL3), regulators of WNK expression and activity, stimulate activity of the $Na^+/Cl^-$ -cotransporter NCC causing hypertension, hyperkalemia and metabolic acidosis [108]. Acidosis in these patients is likely mostly a consequence of impaired renal $K^+$-excretion secondary to NCC overactivity, alternatively, overactivation of pendrin-mediated bicarbonate secretion has been suggested as major mechanism [109].

***Gene defects associated with alkalosis***

Several tubulopathies can be associated with secondary alkalosis such as in patients with Bartter syndrome where alkalosis is thought to be the consequence of the adaptive upregulation of proximal tubular transport pathways in response to volume contraction [110]. Mutations in pendrin (SLC26A4) underlie Pendred syndrome characterized by sensorineural deafness, goiter and hypothyrodism [111]. As discussed earlier, pendrin is involved in the renal defence mechanisms against alkalosis and involved in chloride reabsorption. Whether absence of pendrin predisposes or even causes metabolic alkalosis in humans has remained an open question. A case report from a patient with Pendred syndrome and liver disease and an episode of prolonged vomiting demonstrated severe alkalosis [112].

## Acquired inability of renal acid excretion

### *CKD*

Metabolic acidosis is found in a majority of patients with advanced stages of CKD and a GFR below 20 - 30 ml/min. Plasma bicarbonate levels are reduced to 12-22 mM [113]. Besides the problems associated with chronic metabolic acidosis such as loss of bone density and muscle wasting, metabolic acidosis itself may promote progression of kidney disease as indicated both from animal studies as well as from recent interventional studies demonstrating the beneficial effect of alkalinizing therapies on further loss of kidney function [114-118]. The capacity to maximally acidify urine is preserved in these patients whereas the excretion of ammonium is reduced and clearly inappropriate considering the degree of metabolic acidosis [113]. Studies in rodent models suggest that progression of kidney disease causes reduced expression of enzymes and transporters involved in ammoniagenesis and ammonium excretion [119,120]. Whether only reduced nephron mass or specific dysregulation of components of the ammoniagenic pathway and acid-excretory proteins are responsible for reduced ammonium excretion requires final clarification.

### *Sjögren's syndrome*

Sjögren syndrome is an autoimmune disorder that can affect in about 30 % of patients also renal functions and cause hypokalemic non-anion gap hyperchloremic acidosis with impaired distal renal tubular acidification [121-123]. In a few patients, autoantibodies cross-reacting with intercalated cells have been reported suggesting that antibody-mediated events may impair intercalated cell function [124]. In an

unpublished small series of histological analyses of kidneys from patients with Sjögrens syndrome and dRTA we did not detect any staining for markers of type A intercalated cells suggesting that these cells are either absent or severely impaired (Mohebbi, Walsh, Devuyst, Unwin, Ronco, Wagner, unpublished data). Likewise, absence of $H^+$-ATPase and/or AE1 staining in kidneys from patients with Sjögren's syndrome had been previously reported [73,122]. Also, a case of patient with hypokalemic dRTA with autoimmune atrophic gastritis and hypothyroidism, autoantibodies were detected reacting with intercalated cells but the antigen(s) have remained unknown [125]. Moreover, the kidney biopsy from the patients had no detectable type A intercalated cells which may explain the severe dRTA [125].

**Outlook**

Our understanding of the molecular and cellular mechanisms underlying the kidneys ability to maintain and regulate acid-base balance have advanced much over the past decades. Identification of genes mutated in human disorders of acid-base homeostasis together with the rise of transgenic mouse models have been instrumental to demonstrate the identity and relevance of various pathways. Nevertheless, gaps in our understanding remain and only a few are briefly mentioned here. First, one complex of questions relates to the identity and function of sensors of acid-base status. Several molecules have been identified that can sense changes in intra- and/or extracellular concentrations of protons or bicarbonate including the G protein-coupled receptors OGR1, GPR4 and TDAG8 [7,126,127], the tyrosine receptor kinase Insulin-receptor-related receptor (IRRR) and the receptor-type protein tyrosine phosphatase gamma (RPTP$\gamma$)[128] , the soluble adenylyl cyclase (sAC)[129,130], or the tyrosine kinase Pyk2 [131,132]. Even though it is likely that an essential function such as pH-sensing and regulation does not entirely depend on a single pathway, none of these pathways

seems to play a critical role for the kidneys function in acid-base homeostasis. Clearly, the relative relevance and physiological function of these pathways and molecules has only started to emerge. Second, the interplay of various organs in regulating acid-base balance may deserve more attention. How is the function of organs such as brain, kidney, liver, muscle and bone coordinated ? Are systemic changes in pH sufficient to trigger coordinated responses ? Which other signals are involved ? Third, while a majority of patient cases of pRTA and dRTA can be explained based on identifiable gene mutations, a substantial number of patients with inherited forms of RTA remains with unknown underlying genetic defect(s). One can expect that full genome sequencing together with functional studies on mutated candidate genes will solve many of these cases. Fourth, in acquired syndromes of RTA (i.e. in CKD, Sjögren's syndrome), the cellular and molecular mechanisms have only partly been addressed. While loss of nephron mass plays an important role in reduced ammoniagenesis in CKD, the role of inflammation has not been examined in much detail. Fifth, the impact of acidosis on progression of CKD is an interesting question with a high clinical potential. While several clinical trials are on-going and aim to provide further evidence for the therapeutic potential of alkali supplementation, the underlying mechanisms how acidosis accelerates progression of CKD remain to be further defined. Elucidation of molecular mechanisms may yield novel therapeutic targets. Last, acid-base homeostasis and systemic as well as local inflammation is a mostly unchartered area. The association of local inflammation and hypoxia and acidosis is well documented and acidic pH is involved in recruiting and /or activating inflammatory cells. Whether similar processes are operative during systemic acidosis is not well studied but has major implications for advanced stages of CKD and possibly also for patients with inherited forms of RTA.

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## Figure legends

### Table 1: Molecular genetics of renal tubular acidosis

Summary of genes with known mutations causing distinct forms of renal tubular acidosis. AR autosomal recessive, AD autosomal dominant, XLR X-linked recessive

### Figure 1: Proximal mechanisms of bicarbonate transport

Reabsorption of bicarbonate in the proximal tubule is initiated by active proton secretion via the luminal $Na^+/H^+$-exchanger NHE3 and $H^+$-ATPases. Protons and bicarbonate react to form $CO_2$ and $H_2O$, catalyzed by membane-bound carbonic anhydrase IV (CAIV). $CO_2$ diffuses into proximal tubular cells and is hydrated facilitated by cytosolic carbonic anhydrase II (CAII) to yield $HCO_3^-$ and $H^+$. Whereas $H^+$ can recycle across the luminal membrane, $HCO_3^-$ is transported to blood by the basolateral $Na^+/HCO_3^-$-cotransporter NBCe1. Some luminal $HCO_3^-$ may be directly reabsorbed by the apically located $Na^+/HCO_3^-$-cotransporter NBCn2. Mutations in NBCe1 can cause pRTA, mutations in CAII give rise to a mixed pRTA and dRTA phenotype.

### Figure 2: Proximal ammoniagenesis and tubular ammonium handling

***Upper panel:*** Ammoniagenesis in the proximal tubule. Glutamine is extracted from blood involving SNAT3 and LAT2 transporters and converted to α-ketoglutarate by mitochondrial phosphate-dependent glutaminase (PDG) and glutamate dehydrogenase (GDH) thereby releasing $NH_3$. α-ketoglutarate is the substrate for renal gluconeogenesis and $NH_3$ mostly combines with protons to form $NH_4^+$ which is released into urine. SNAT3: sodium dependent amino acid transporter SLC38A3, LAT2 type L amino acid transporter, NHE3: sodium/proton antiporter isoform 3

SLC9A3, NBCe1: sodium bicarbonate cotransporter SLC4A4, PDG: phosphate-dependent glutaminase, GDH: glutamate dehydrogenase, αKG: α-ketoglutarate, TCA: tricarboxylic acid (TCA) cycle, PEPCK: phosphoenolpyruvate carboxykinase.

***Lower panel:*** Ammonium transport along the nephron. $NH_4^+$ released into urine in the proximal tubule is partly reabsorbed in the thick ascending limb of the loop of Henle and accumulated with a cortico-medullary gradient in the interstitium. $NH_3$ is secreted into urine along the collecting duct and is ultimately excreted as $NH_4^+$.

**Figure 3: Acid-base handling by collecting duct intercalated cells, indicate mutations**

Schematic of major transport pathways in type A and B intercalated cells. Type A intercalated cells (upper panel) secrete $H^+$ via apical $H^+$-ATPases and $H^+/K^+$-ATPases into urine where they are buffered by either by $NH_3$ or titratable acids (TA). Protons are generated from $CO_2$ and $H_2O$ catalyzed by cytosolic carbonic anhydrase II (CAII) yielding also $HCO_3^-$ to be released into blood by basolateral $Cl^-/HCO_3^-$-exchanger AE1. Type A intercalated cells, together with other collecting duct cells take up $NH_4^+$ via NKCC1 and $Na^+/K^+$-ATPases and $NH_3$ via RhCG and RhBG transporters. $NH_3$ is secreted into urine by luminal RhCG transporters. Type B intercalated cells express luminally the $Cl^-/HCO_3^-$-exchanger pendrin that either works in parallel with luminal $H^+$-ATPases to mediate net chloride absorption or works together with basolateral $H^+$-ATPases to allow for net secretion of $HCO_3^-$ and absorption of $Cl^-$.

**Figure 4: Mutations in intercalated cells causing dRTA**

Mutations in either the SLC4A1 (AE1) transporter or the a4 or B1 isoforms of the $H^+$-ATPase subunits can cause dRTA. Also mutations in the FOXI1 transcription factor

controlling expression of AE1, CAII, and the B1 and a4 $H^+$-ATPase subunits causes dRTA. The insert shows a model of the $H^+$-ATPase indicating the localization of the B (either B1 or B2) and a (a1-a4 isoforms) subunits.

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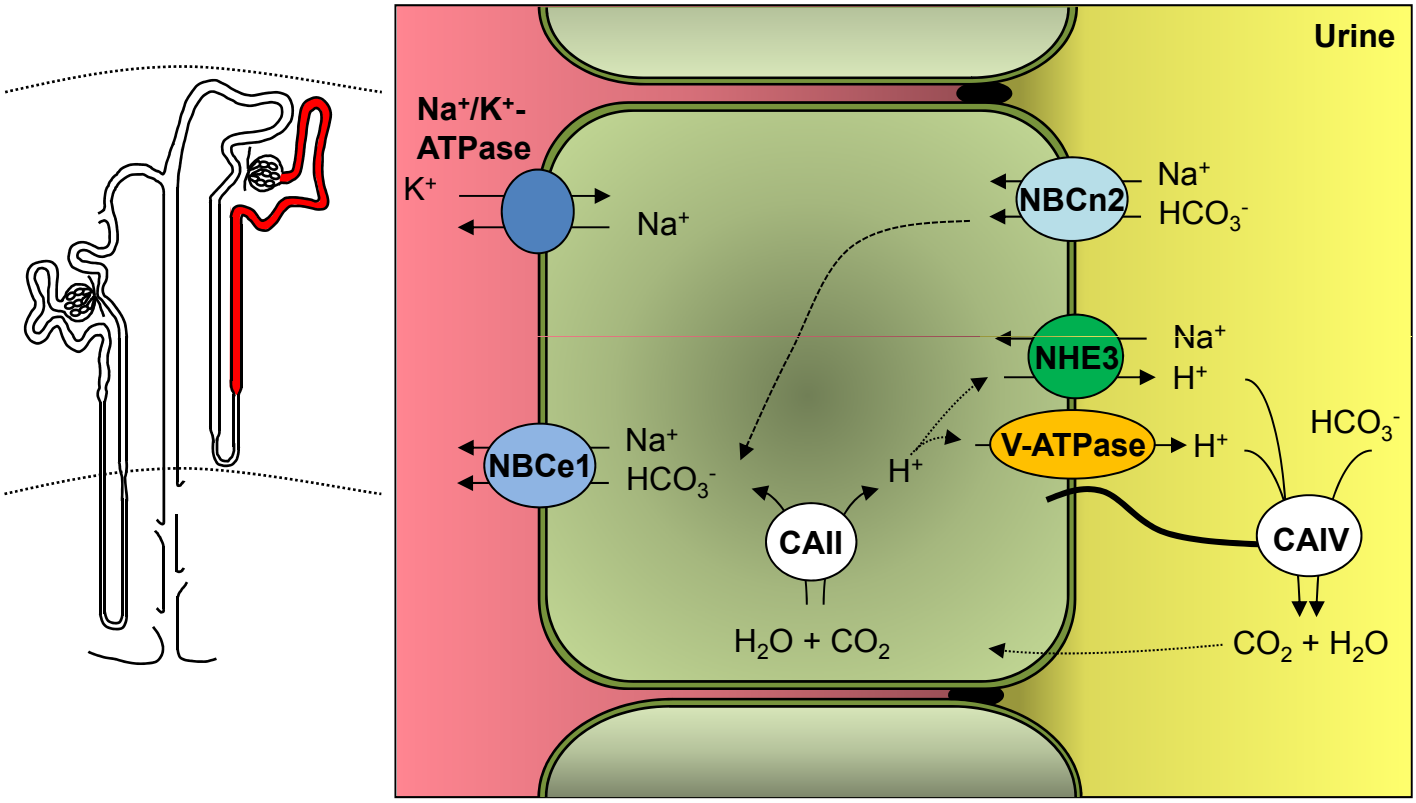
**Table 1: Molecular genetics of renal tubular acidosis**

| Type RTA | Gene | Protein | Inheritance | OMIM[1] |
|---|---|---|---|---|
| **Type I** | | | | |
| | SLC4A1 | AE1 $Cl^-/HCO_3^-$ anion exchanger 1 | AD/AR | # 611590 |
| | ATP6V1B1 | B1 $H^+$-ATPase subunit | AR | # 267300 |
| | ATP6V0A4 | a4 $H^+$-ATPase subunit | AR | # 602722 |
| | FOXI1 | Forkhead box I1 | AR | # 600791[2] |
| | WDR72 | WDR72 | AR | # 613211 |
| **Type II** | | | | |
| | SLC4A4 | $Na^+$-$HCO_3^-$-cotransporter NBCe1 | AR | # 604278 |
| | CLCN5 | $Cl^-/H^+$-antiporter | XLR | # 300009, # 10468 |
| | OCRL | Oculocerebrorenal syndrome Lowe | XLR | # 309000 |
| | SLC2A2 | GLUT2 | AR | # 227810 |
| | CTNS | Cystinosin | AR | # 219800 |
| | EHHADH | Enoyl-CoA-hydratase:3-hydroxyacyl-CoA dehydrogenase | AD | # 615605 |
| | ATP7B | $Cu^{2+}$-ATPase | AR | # 277900 |
| | FAH | Fumarylacetoacetate hydrolase | AR | # 276700 |
| | TAT | Tyrosine aminotransferase | AR | # 276600 |
| | HPD | 4-hydroxyphenylpyruvate dioxygenase | AR/ AD | # 276710 # 140350 |
| | GALT | Galactose-1-phosphate uridylyl-transferase | AR | # 230400 |
| **Type III** | | | | |
| | CAII | Carbonic anhydrase II | AR | # 259730 |
| **Type IV** | | | | |
| | CYP11B2 | 11-beta-hydroxylase, aldosterone synthase | AR | # 202010 |
| | NR3C2 | Mineralocorticoid receptor | AD | # 177735 |
| | SCNN1A | $\alpha$ subunit epithelial $Na^+$ channel | AR | # 264350 |
| | SCNN1B | $\beta$ subunit epithelial $Na^+$ channel | AR | # 264350 |
| | SCNN1G | $\gamma$ subunit epithelial $Na^+$ channel | AR | # 264350 |
| | WNK1 | With No Lysine kinase 1 | AD | # 614492 |
| | WNK4 | With No Lysine kinase 4 | AD | # 614491 |
| | KLHL3 | Kelch-like 3 | AD/AR | # 614495 |
| | CUL3 | Cullin-3 | AD | # 614496 |

[1] Online Mendelian Inheritance in Man: https://www.omim.org/

[2] no distinct OMIM number has been assigned to FOXI1 mutations, the number refers to a phenotype

# Figure 1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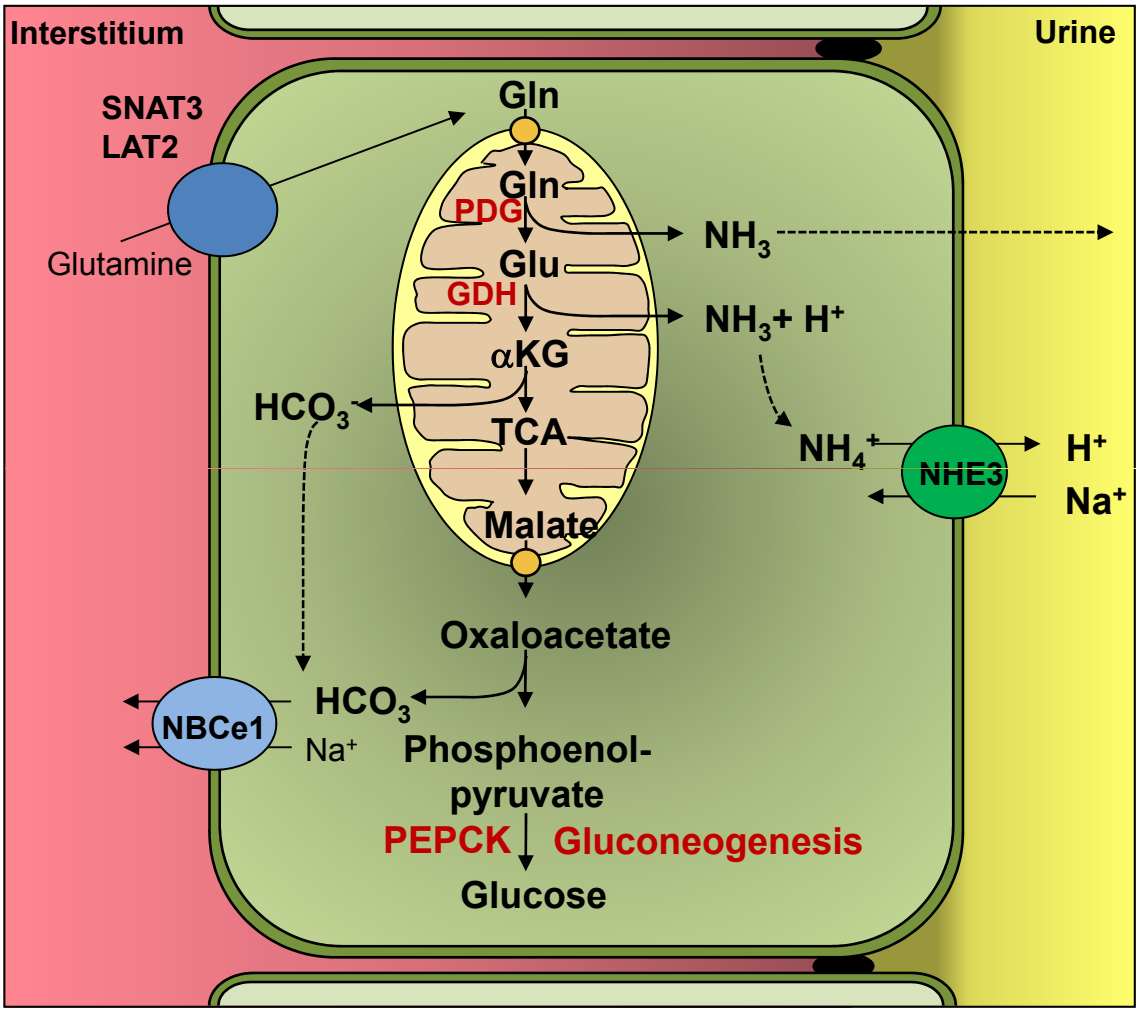
Na+/K+-
ATPase
K+
Na+
NBCn2
Na+
HCO3-
NHE3
Na+
H+
NBCe1
Na+
HCO3-
V-ATPase
H+
HCO3-
H+
CAII
CAIV
H2O + CO2
CO2 + H2O

# Figure 2


Interstitium
Urine
SNAT3
LAT2
Glutamine
Gln
Gln
PDG
$NH_3$
Glu
GDH
$NH_3$+ $H^+$
αKG
$HCO_3^-$
TCA
$NH_4^+$
NHE3
$H^+$
$Na^+$
Malate
Oxaloacetate
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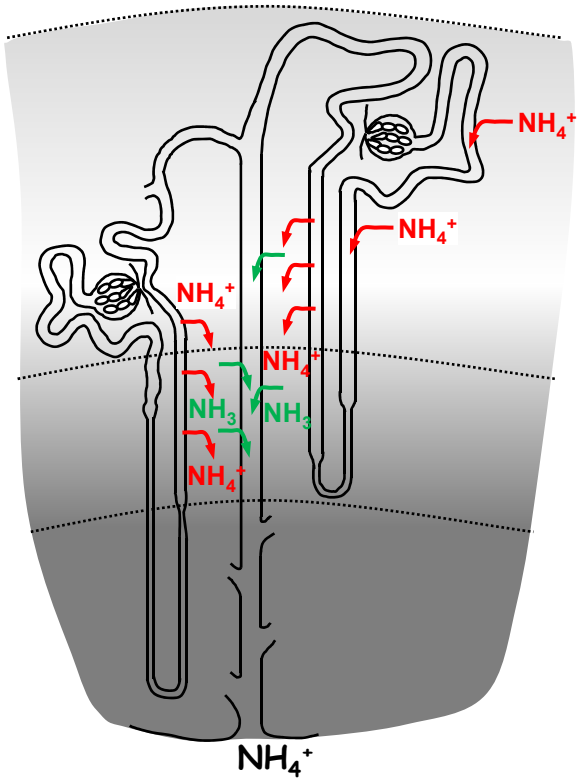
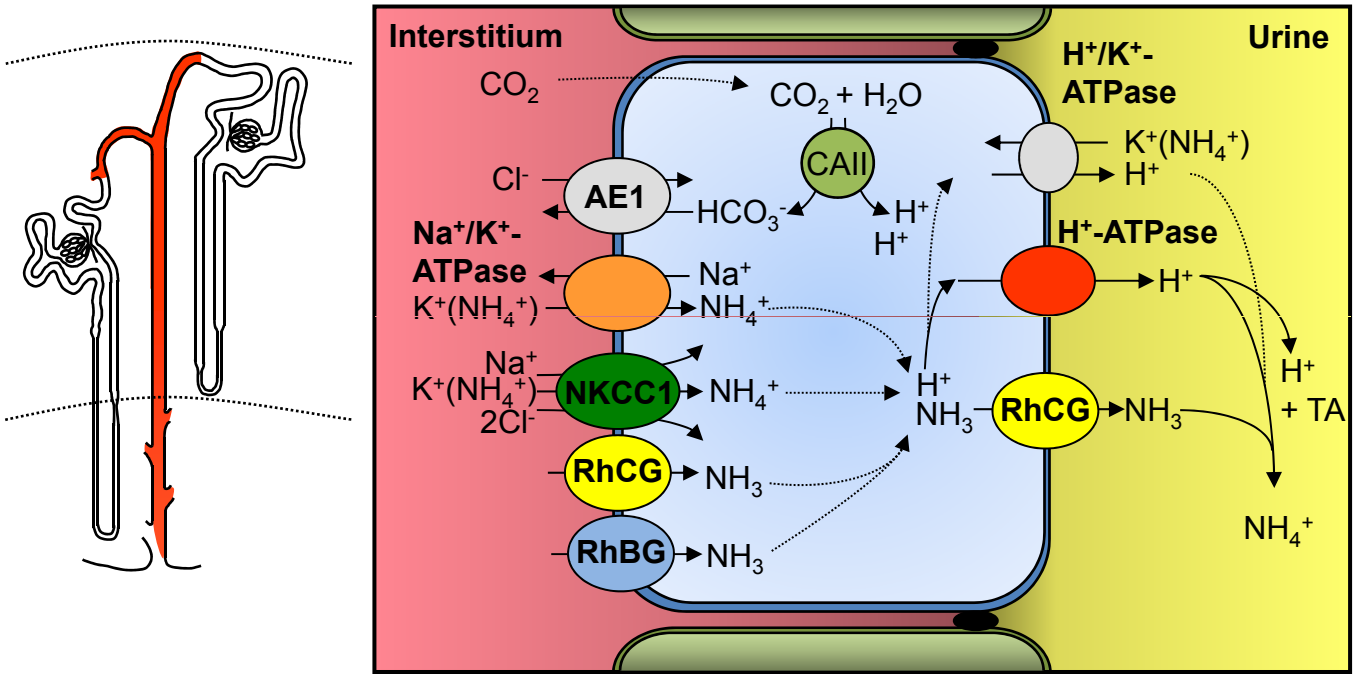
$Na^+$
Phosphoenol-
pyruvate
PEPCK
Gluconeogenesis
Glucose

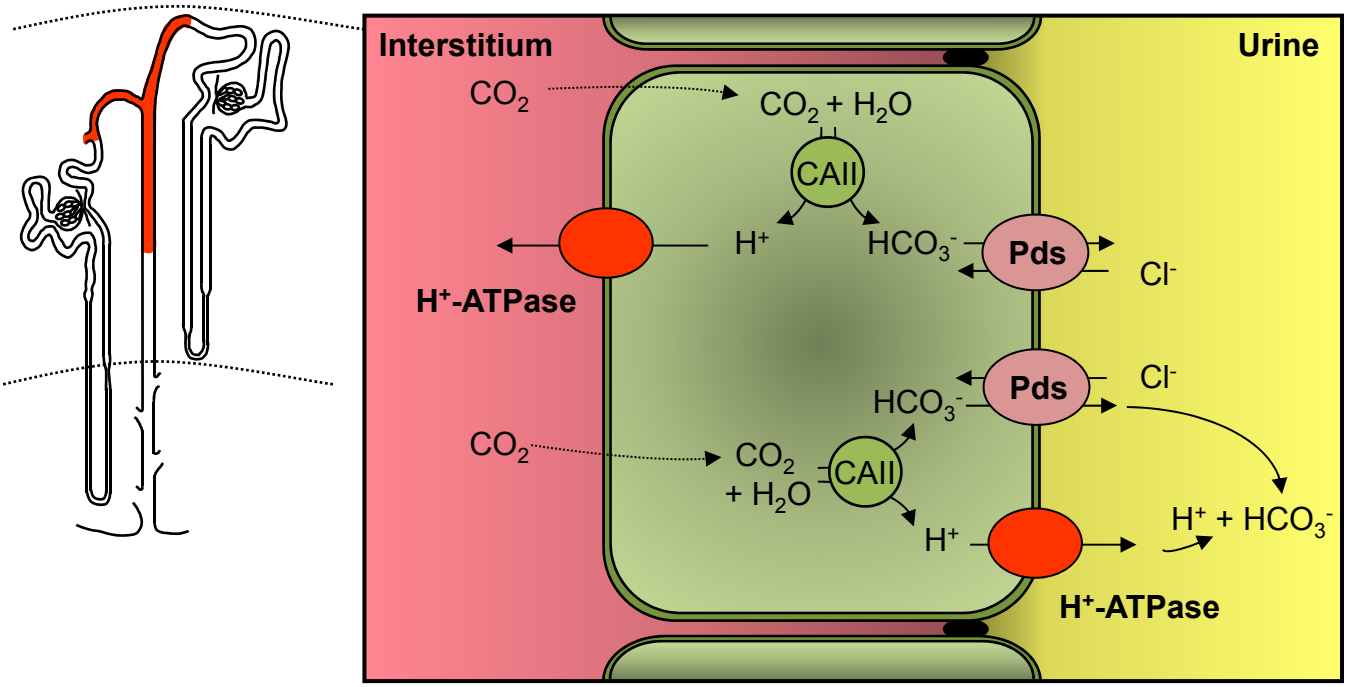

$NH_4^+$
$NH_4^+$
$NH_4^+$
$NH_4^+$
$NH_3$
$NH_3$
$NH_4^+$
$NH_4^+$

# Figure 3

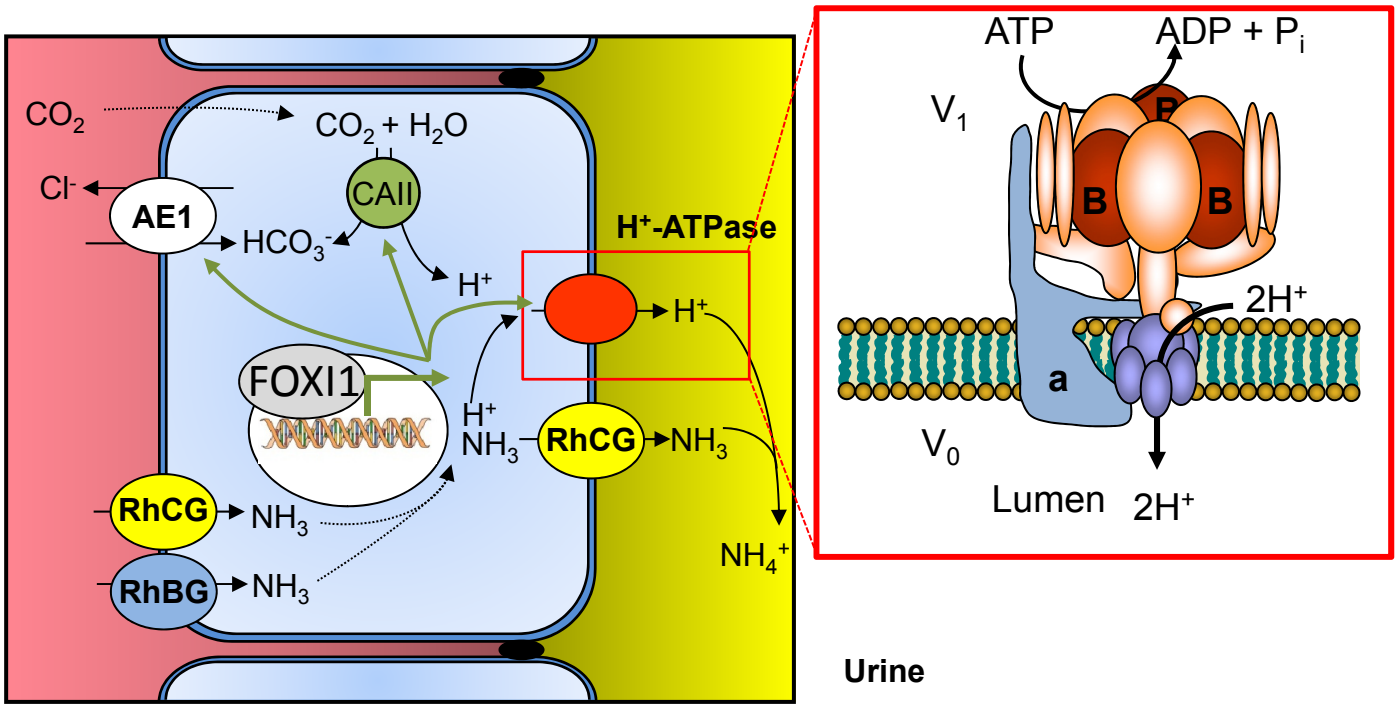
## Type A Intercalated cells




## Type B Intercalated cells



# Figure 4


CO2
CO2 + H2O
Cl-
AE1
CAII
HCO3-
H+
H+-ATPase
H+
FOXI1
H+
NH3
RhCG
NH3
RhCG
NH3
RhBG
NH3
NH4+
Urine
ATP
ADP + Pi
V1
B
B
B
2H+
a
V0
Lumen
2H+